

molecules on its surface<sup>17</sup>. These cells bound equivalent amounts of the peptide regardless of the presence of TSST-1 (Fig. 4a). That TSST-1 was actually bound to these cells is demonstrated in Fig. 4b. The data are shown as separate fluorescence histograms for clarity; two-colour fluorescence contour plots revealed a single homogeneous population of cells positive for both peptide and TSST-1 binding. Figure 4c demonstrates similar findings for a stable L-cell transfectant expressing DR1. The quantity of class II on these cells is about one-tenth that of the B cells. The level of peptide binding was correspondingly lower. Again, pre-incubation of the cells with TSST-1 did not affect the level of peptide binding. The binding of TSST-1 to these cells is shown in Fig. 4d. An L-cell transfectant expressing equivalent amounts of DPw2 did not bind either the biotinylated peptide or TSST-1 (Fig. 4e,f).

It has been estimated that ~1% of the surface DR molecules will bind the haemagglutinin peptide under the conditions used<sup>14</sup>. Presumably, the toxin is unable to distinguish those class II molecules that will eventually bind the biotinylated peptide from those that do not. The simultaneous binding of TSST-1 and peptide to the same DR molecule is then most consistent with the model of toxin contacting both the class II and the TCR outside of the peptide-binding site.

There is some evidence for a functional interaction between the  $\alpha$ -chain of class II and the  $\beta$ -chain of the T cell receptor. The maximal response of murine T cells to a mitogen from *Mycoplasma arthritidis*, which is predominantly by cells bearing V $\beta$ 6 and V $\beta$ 8 TCRs, requires the presence of the I-E  $\alpha$ -chain<sup>18</sup>. L cells expressing either the E $\alpha$ -E $\beta$  or E $\alpha$ -A $\beta$  murine class II molecules were far better stimulators of purified T cells in the presence of mycoplasma mitogen than were fibroblasts expressing A $\alpha$ -A $\beta$ . Also, the expression of an E $\alpha$  transgene in H-2<sup>b</sup> mice that do not normally express E $\alpha$  leads to the ability of the mitogen to stimulate splenocytes *in vitro*. This is consistent with the hypothesis that this mitogen requires the presence of E $\alpha$  for binding to class II. In addition, Berg, *et al.*<sup>19</sup> have studied the selection of the T-cell repertoire in TCR transgenic mice. Mice that express transgenes for particular TCR  $\alpha$ - and  $\beta$ -chains have strict requirements for the class II haplotypes that will lead to positive selection of the transgenic TCR. However, mice that express the transgenic TCR  $\beta$ -chain in association with a variety of endogenous TCR  $\alpha$ -chains are selected by several I-E haplotypes (all of which share a nonpolymorphic class II  $\alpha$ -chain), suggesting that the TCR V $\beta$  region interacts with the physical, interaction between the  $\alpha$ -chain of I-E and the V $\beta$  region of the TCR. The finding that the high-affinity binding of TSST-1 to HLA-DR requires the  $\alpha$ 1 domain is consistent with this hypothesis, and suggests that there are contact points between the DR  $\alpha$ -chain and the V $\beta$  domain of the TCR.

## Empty MHC class I molecules come out in the cold

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MAJOR histocompatibility complex (MHC) class I molecules present antigen by transporting peptides from intracellularly degraded proteins to the cell surface for scrutiny by cytotoxic T cells. Recent work suggests that peptide binding may be required for efficient assembly and intracellular transport of MHC class I molecules<sup>1</sup>, but it is not clear whether class I molecules can ever assemble in the absence of peptide. We report here that culture of the murine lymphoma mutant cell line RMA-S at reduced temperature (19–33 °C) promotes assembly, and results in a high level of cell surface expression of H-2 $\beta$ / $\beta$ <sub>2</sub>-microglobulin complexes that do not present endogenous antigens, and are labile at 37 °C. They can be stabilized at 37 °C by exposure to specific peptides known to interact with H-2K<sup>b</sup> or D<sup>b</sup>. Our findings suggest that, in the absence of peptides, class I molecules can assemble but are unstable at body temperature. The induction of such molecules at reduced temperature opens new ways to analyse the nature of MHC class I peptide interactions at the cell surface.

A mouse mutant lymphoma cell line, RMA-S, expresses at the cell surface <10% of the amount of class I molecules compared with RMA mutagenized but unselected control line<sup>2,3</sup>—and is unable to present endogenous antigens<sup>4,5</sup>. Rates of synthesis of class I heavy and light chains are normal in RMA-S, and analysis of RMA-S-L-cell hybrids excludes the possibility that the defect involves a mutation in the H-2 heavy or light chain of RMA-S<sup>6</sup>.

In an experiment originally designed to examine the contribution of the various protein breakdown routes<sup>7</sup> to reduced surface expression in RMA-S, cells were pulse-labelled at 37 °C and chased at 26 °C. At this temperature, essentially no breakdown of class I molecules of the RMA wild-type line occurred, and glycan modifications were slower compared with chase at 37 °C (Fig. 1a). Breakdown of class I molecules was also decreased at 26 °C for RMA-S but, surprisingly, extensive modifications of N-linked glycans were observed (Fig. 1a).

Culture of RMA-S at temperatures ranging from 8–33 °C (optimum 23–31 °C) also led to a marked increase in the expression of K<sup>b</sup> and D<sup>b</sup> at the cell surface (Fig. 2a). Expression increased linearly with time (shown for 22 °C), up to 28 h (Fig. 2b). Culture of RMA-S at 26 °C for 24 h (notation, RMA-S (26 °C)) was adopted as the standard treatment, unless stated otherwise. The class I molecules that appear at the cell surface at low temperature seem to be folded correctly, and are associated with  $\beta$ <sub>2</sub>-microglobulin. For both D<sup>b</sup> and K<sup>b</sup>, a parallel increase in the binding of monoclonal antibodies to epitopes on the  $\alpha$ 1,  $\alpha$ 2 or  $\alpha$ 3 domains of the heavy chain, as well as to  $\beta$ <sub>2</sub>-microglobulin was observed (Fig. 2c). Comparison of surface-labelled material of RMA-S (26 °C) and RMA-S (37 °C) immunoprecipitated with either anti-H-2<sup>b</sup> serum or the confor-

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mation-independent anti-K<sup>b</sup> exon 8 antibody (a gift from Dr B. Barber) reveals the existence of class I molecules on RMA-S (37 °C) that are not recognized by the anti-H-2<sup>b</sup> serum (possibly reflecting the population of H-2 molecules that can be stabilized by addition of peptide at 37 °C). But there is a significant increase in the amount of material recovered with both antisera on culture at reduced temperature (data not shown). The effect of low temperature on class I expression on RMA-S differs from the effect of peptides added at 37 °C<sup>1</sup>. Low temperature increases the expression of both D<sup>b</sup> and K<sup>b</sup>, whereas peptides have predominantly allele-specific effects<sup>1</sup>. The two effects are additive during the first 6 h of incubation at 26 °C (data not shown). The effect of low temperature on class I expression was also observed, but to a lesser extent, on RMA under identical conditions (Fig. 2b).

RMA-S has been reported to be incapable of presenting internally derived antigens to H-2-restricted cytotoxic T lymphocytes (CTL) specific for viral<sup>2</sup>, minor histocompatibility or tumour antigens<sup>3</sup>. RMA-S (26 °C) remained resistant to H-2<sup>b</sup>.

restricted CTL directed against minor histocompatibility antigens (Fig. 3a), despite a 5- to 10-fold increase in H-2K<sup>b</sup> and D<sup>b</sup> expression at the cell surface measured in parallel by cytofluorimetry (data not shown). Similarly, the RMA-S (26 °C) cells remained resistant to a H-2D<sup>b</sup>-restricted influenza-specific CTL clone after virus infection at 26 °C (Fig. 3b). But when tested with a H-2D<sup>b</sup>-restricted peptide, NP 366-379<sup>1</sup>, RMA-S (26 °C) was 100-fold more susceptible to lysis than RMA-S (37 °C) in terms of the peptide concentration required to induce 50% killing (Fig. 3c). Although RMA-S (26 °C) had half the H-2D<sup>b</sup> expression of RMA (37 °C), RMA-S (26 °C) could be sensitized at a far lower concentration of peptide than RMA (37 °C). Note that the peptide titration curve shifted also for RMA (26 °C).

We propose that the class I molecules exported to the cell surface at 26 °C in RMA-S lack peptide in their antigen-binding pocket. Our previous experiments suggested that peptide is required for assembly and stabilization of class I molecules at 37 °C in RMA-S<sup>1</sup>. To test whether the class I molecules induced in RMA-S (26 °C) were unstable at physiological temperature,

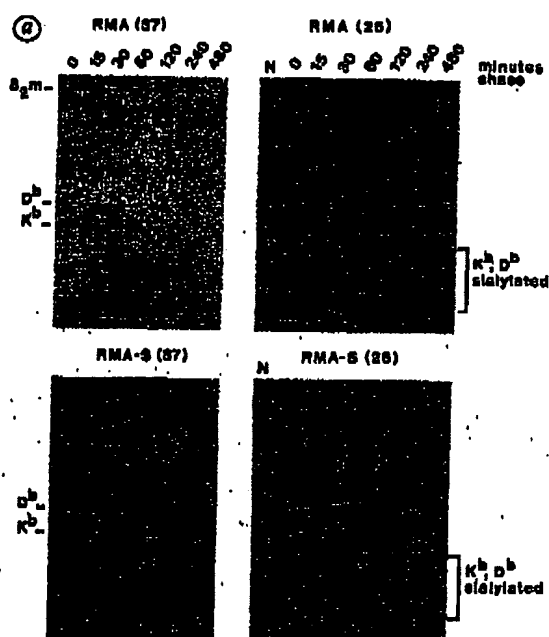
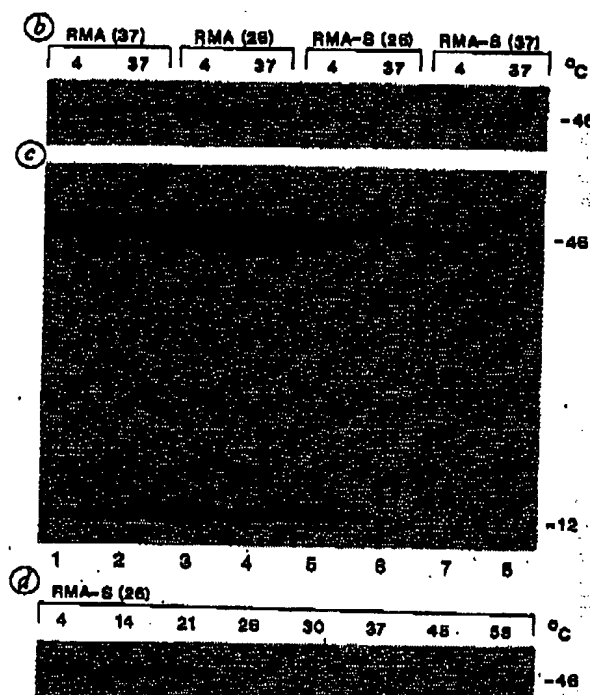


FIG. 1 Post-translational modifications and stability of class I molecules induced at low temperature. a RMA and RMA-S were cultured and metabolically labelled for 10 min at 37 °C, chased for the times indicated at either 26 °C or 37 °C, and lysed. Class I molecules were immunoprecipitated and analysed on 1D-IEF gels. The position of  $\beta_2$ -microglobulin ( $\beta_2$ m) and non-elated H-2K<sup>b</sup> and D<sup>b</sup> are indicated on the left, elyated forms of K<sup>b</sup> and D<sup>b</sup> by the brackets on the right. Class I molecules showed a significantly prolonged half life at 26 °C in comparison with 37 °C for both RMA and RMA-S. Importantly, the extent of terminal glycan modifications (elyation), indicating transport through the trans-Golgi, was higher in RMA-S chased at 26 °C than at 37 °C. b, c RMA and RMA-S were cultured at either 37 °C or 26 °C, surface-labelled by iodination and lysed. Lysates were incubated at the temperatures indicated. Class I molecules were then immunoprecipitated and analysed by SDS-PAGE. Note the disappearance of anti-H-2 reactive material of RMA-S (26 °C) on incubation of the lysate at 37 °C (only the heavy chain region of the gel is shown in b). The small amounts of H-2 that are expressed on the surface of RMA-S (37 °C) exhibit similar thermolability (a is a longer exposure of b). d incubation of RMA-S (26 °C) lysates was performed at different temperatures, as indicated. Note the reduction in immunoreactive H-2 class I molecules after incubation at temperatures higher than 26 °C.

METHODS. a Ten million cells were pulse-labelled with 500  $\mu$ Ci [<sup>35</sup>S]methio-



nine (1,200 Ci mol<sup>-1</sup>, Amersham) for 10 min at 37 °C and chased in the presence of excess of cold methionine for different periods at either 26 °C or 37 °C (ref. 14). After lysis, free  $\beta_2$ m was removed by immunoprecipitation using an anti- $\beta_2$ m serum (K287, ref. 15). Specific immunoprecipitation used a rabbit anti-mouse H-2 serum (a gift from L. Rask, Uppsala; ref. 16 and S. Nathanson, New York) and analysed on 1D-IEF as described (ref. 17 and H.G.L., M. Oudshoorn-Snoek, M. G. Masucci and H.L.P., manuscript submitted). The anode is at the bottom. N, immunoprecipitates prepared with normal mouse serum as control. b-d Cells were cultured for 48 h at 26 °C or 37 °C in RPMI-1440 medium, fetal calf serum (FCS). Five million cells were surface-labelled by lactoperoxidase-catalysed iodination as described<sup>28</sup>. Cells were lysed on ice in 1 ml lysis buffer (1% Triton X-100, 140 mM NaCl, 10 mM Tris buffer, pH 7.8, 1 mM phenylmethylsulphonyl fluoride, 1  $\mu$ g ml<sup>-1</sup> trypsin inhibitor, 1  $\mu$ g ml<sup>-1</sup> leupeptin, 30 ml of trypsin inhibitor ml<sup>-1</sup> aprotinin (Sigma)). The lysates were incubated at the temperature indicated for 1 h and transferred to 0 °C. H-2 class I were immunoprecipitated with rabbit anti-H-2 serum (see above) as described<sup>27</sup>, and analysed by SDS-PAGE on a 12% gel. Relative molecular mass standards ( $M_r \times 10^{-3}$ ) are to the right.

# LETTERS TO NATURE

H-2 expression was examined after shifting the cells to 37 °C. Brefeldin A (BFA) was added to prevent further transport of class I molecules from the endoplasmic reticulum to the cell surface<sup>7,8</sup>. Within 1 h, more than half the D<sup>b</sup> class I molecules induced at 26 °C had disappeared from the cell surface (Fig. 4a). After 4 h at 37 °C only trace amounts of induced K<sup>b</sup> and D<sup>b</sup> molecules remained (Fig. 4b). A similar decrease was observed for K<sup>b</sup> and D<sup>b</sup> in BFA-treated RMA-S (37 °C) cells (Fig. 4a, 4b), in agreement with the rapid decay of biosynthetically labelled class I molecules in RMA-S (37 °C). By contrast, more than 80% of class I molecules was still detectable at the surface after 2 h, in BFA-treated RMA (37 °C) cells and their half-life was >6 h as measured by cytofluorimetry (data not shown).

Significant amounts of surface-iodinated H-2 class I molecules, comparable to the amounts seen in RMA (37 °C), could be immunoprecipitated from RMA-S (26 °C) (compare lanes 1 and 3, Fig. 1b, c). When lysates were incubated at 37 °C, a complete loss of immunoreactive class I molecules of RMA-S (26 °C) or (37 °C) was observed (compare lanes 5 and 6; Fig. 1b-d). For RMA-S lysates, but not for RMA lysates, there was a sharp drop in quantity of H-2 recovered, with increasing temperature (Fig. 1d). The loss of H-2 class I molecules in the lysates from RMA-S was not due to proteolysis, as judged by the continued presence of H-2K<sup>b</sup> and D<sup>b</sup> heavy chains revealed by two-dimensional isoelectric focusing (IEF)/SDS-PAGE analysis of the total lysate (data not shown).

If the class I complexes induced at the RMA-S cell surface at low temperature are unstable at 37 °C because they are devoid of peptides, addition of class I-binding peptides to BFA-treated cells before transfer to 37 °C, should stabilize them. In accordance with their known restriction specification, this was indeed the observed result for epitopes presented in the context of H-2K<sup>b</sup> and D<sup>b</sup> (Fig. 4b, c), at concentrations of peptide, previously shown to restore H-2 expression in RMA-S (37 °C). This peptide-mediated stabilization could be confirmed by biochemical analysis of surface-labelled RMA-S (26 °C)<sup>9</sup>.

In summary, culture of RMA-S at low temperature induces MHC class I molecules at the cell surface that: (1) undergo post-translational modifications characteristic of passage through the trans-Golgi (Fig. 1a); (2) are associated with  $\beta_2$ -microglobulin at the cell surface and express conformational epitopes found in properly folded class I molecules (Figs 1c and 2); (3) are unstable at 37 °C (Figs 1b-d and 4a-b); (4) fail to present internally derived antigens to CTL (Fig. 3a, b); (5) present exogenous peptides more efficiently than class I molecules of the wild-type line cultured at 37 °C (Fig. 3c), and (6) are stabilized by exogenous peptides in an allele-specific manner (Fig. 4b, c). The most simple interpretation is that MHC class I molecules devoid of internally derived peptides can reach the cell surface at the reduced temperature.

We propose the following explanation for our findings. Under normal conditions, the MHC class I subunits will combine with peptide early in biosynthesis. If they fail to do so, a heterodimer may fail to form or, if devoid of peptide, have a high probability of dissociating or displaying a grossly altered conformation at 37 °C. We surmise that such 'empty', aberrantly folded heavy chains would not be transported further and be rerouted or degraded. That fraction of class I/ $\beta_2$ -microglobulin heterodimers that reaches the cell surface empty at 37 °C can be stabilized by a suitable peptide supplied during experimental procedures. The intrinsic instability of empty class I molecules can be overcome not only by addition of peptide, but also by reducing the temperature. The affinity of the class I heavy chain for  $\beta_2$ -microglobulin is thus sufficiently high at the reduced temperature to allow an increase in net transport of the complex to the cell surface in the absence of peptide.

The findings reported here underscore the role for peptide in maintaining structural integrity of class I molecules, but are consistent with the possibility that much of the increase in H-2

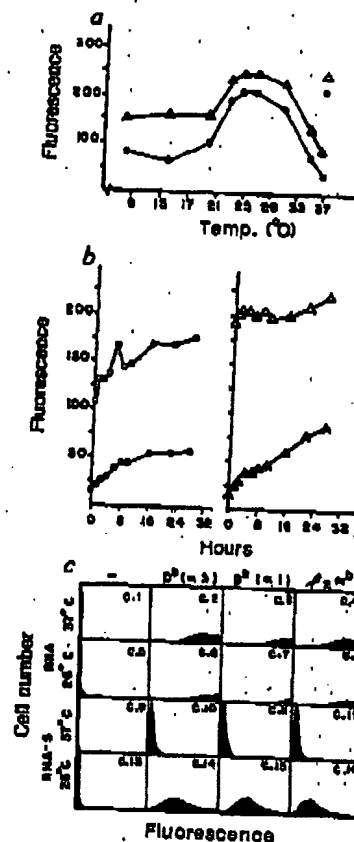


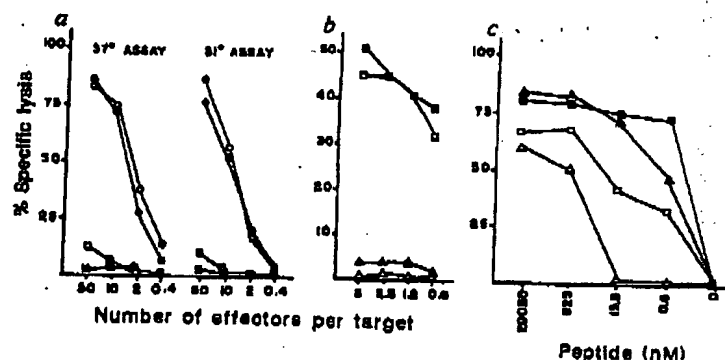
FIG. 2 Induction of cell surface class I molecules at low temperature. a, Staining with monoclonal antibodies against K<sup>b</sup> (28-13-SS; ref. 19) and D<sup>b</sup> (28-14-SS; ref. 20-22) at the cell surface of RMA-S cells cultured for 24 h at different temperatures. Quantitation of K<sup>b</sup> and D<sup>b</sup> expression on the control line RMA cultured at 37 °C is indicated for comparison. ●, RMA-S anti-K<sup>b</sup>; ▲, RMA-S anti-D<sup>b</sup>; ○, RMA anti-K<sup>b</sup>; △, RMA anti-D<sup>b</sup>. On average, in a series of 10 experiments, K<sup>b</sup> and D<sup>b</sup> expression increased to a level corresponding to 40–55% of RMA (37 °C). In occasional experiments the level of expression reached that of RMA (37 °C). b, Kinetics of class I increase at the cell surface measured with monoclonal antibodies against H-2K<sup>b</sup> (28-13-SS) and D<sup>b</sup> (28-14-SS) at 22 °C on RMA-S and RMA. ●, RMA-S anti-K<sup>b</sup>; ▲, RMA-S anti-D<sup>b</sup>; ○, RMA anti-K<sup>b</sup>; △, RMA anti-D<sup>b</sup>. c, H-2D<sup>b</sup> induced at low temperature is associated with  $\beta_2$ m and expresses a conformation-dependent epitope on the  $\alpha 1$  domain. Cell surface staining with monoclonal antibodies 22-14-SS (D<sup>b</sup>,  $\alpha 1$ ; charts C.2, C.8, C.10 and C.14), B22.249 (D<sup>b</sup>,  $\alpha 1$ ; charts C.3, C.7, C.11, C.15; ref. 21-23) and mo- $\beta_2$ m-8 ( $\beta_2$ m allele; charts C.4, C.8, C.12, C.14) of RMA (charts C.1-C.8) and RMA-S (charts C.9-C.14) cultured for 24 h at 26 °C or continuously at 37 °C. Charts C.1, C.5, C.9 and C.13 show background staining with no first (specific) antibody (—). Similar results were obtained for K<sup>b</sup> with the antibodies Y3 ( $\alpha 2$ , ref. 25) and B19.8 (ref. 26) (data not shown).

METHODS. All cells were cultured in RPMI-1440, 10% FCS supplemented with antibiotics. Cells were removed from 37 °C (5% CO<sub>2</sub>), cultured (10<sup>6</sup> cells ml<sup>-1</sup>) in tissue-culture flasks (Falcon, 80 ml) with tightened cap, placed in a heat-adjustable water bath for 24 h at a given temperature unless noted otherwise. Culture at temperatures <8 °C for 24 h or more, usually reduced viability of cells. For cytofluorimetry, 10<sup>6</sup> cells were incubated with 0.1 ml anti-class I monoclonal antibody tissue culture supernatant for 30 min on ice, washed twice in PBS, and then incubated with 0.1 ml fluorescein isothiocyanate (FITC)-conjugated rabbit anti-mouse immunoglobulin (Z108; DAKO, Copenhagen, Denmark) on ice for 30 min, washed twice in PBS, fixed in 1% formaldehyde and analysed on a FACS IV cell sorter. Results from a and b derived from mean linear fluorescence values obtained by FACS analysis. Monoclonal antibodies 28-14-SS and 28-13-SS were obtained from ATCC (Rockville, MD), B22.249 was a gift from G. J. Hammerling, mo- $\beta_2$ m-8 (ref. 27) was a gift from E. A. Boyse.

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FIG. 3 Sensitivity to cytotoxic effector cells after induction of class I molecules at low temperature on RMA and RMA-S. a, RMA-S (26 °C) cells are not recognized by CTL specific for minor histocompatibility antigens in  $^{51}\text{Cr}$  release assay at 37 °C (left chart) or at 31 °C (right chart). Target cells were: ●, RMA (37 °C); ○, RMA (26 °C); ■, RMA-S (37 °C); □, RMA-S (26 °C). Note that this resistance was also observed if the cytotoxicity assay was at 31 °C (Fig. 3a), that is, within the temperature range permissive for high levels of cell surface expression of class I molecules (Fig. 2a). b, Low temperature treated RMA-S cells infected with a recombinant vaccinia that expresses a rapidly degraded C-terminal fragment of influenza NP (IMP-VAC, ref. 28) are also not recognized by the NP-specific CTL clone, F8. Target cells were: □, RMA (37 °C) infected with IMP-VAC; ■, RMA (26 °C) infected with IMP-VAC; △, RMA-S (37 °C) infected with IMP-VAC; ▲, RMA-S (26 °C) infected with IMP-VAC. Uninfected cells were not lysed above 1% after treatment at either temperature (not shown). c, Recognition by clone F8 of RMA and RMA-S after incubation at 26 °C or 37 °C and exposure to decreasing concentrations of peptide NP 365-379 (ref. 1). △, RMA-S (37 °C); ▲, RMA-S (26 °C); □, RMA (37 °C); ■, RMA (26 °C). The CTL clone F8 was used at a killer/target ratio of 5/1 throughout. METHODS. CTL in a were generated by secondary mixed lymphocyte culture across a minor histocompatibility barrier (A by anti-B6) as described<sup>9</sup>.  $^{51}\text{Cr}$ -labelling of targets were performed at 26 °C and 37 °C, respectively. A standard protocol for the  $^{51}\text{Cr}$  release assay was used<sup>9</sup>. Clone F8 in b was isolated from an influenza virus-infected B6 mouse as described<sup>28</sup>. In c it was tested against RMA and RMA-S infected with a recombinant vaccinia that expressed the C-terminal portion of influenza NP (amino acids 327-498) under the control of the vaccinia virus 7.5K promoter<sup>28</sup>. Target cells were incubated at 26 °C or 37 °C for 24 h, collected and infected with 10 plaque-forming units per cell of recombinant vaccinia, and labelled with  $^{51}\text{Cr}$  at the



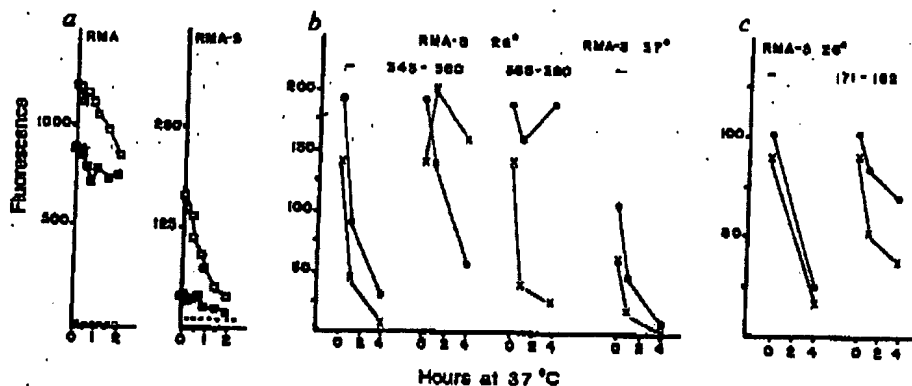
same temperature for 90 min. After washing, the cells were again resuspended at 26 °C or 37 °C for 4 h to allow for synthesis of vaccinia-encoded proteins. Cells were again washed and set up in a 4 h  $^{51}\text{Cr}$  release assay as described at 37 °C (ref. 29) with CTL clone F8 at the killer/target cell (K/T) ratios shown. Surface expression of D<sup>p</sup> measured with the monoclonal antibody B22.249 (mean fluorescence) measured after incubation at the different temperatures was 20, RMA-S (37 °C); 44, RMA-S (26 °C); 277, RMA (37 °C); 328, RMA (26 °C). In a, RMA-S and RMA were collected after 24 h at 26 °C or 37 °C, labelled with  $^{51}\text{Cr}$  and incubated with peptide NP 365-379 (ref. 29) at the same temperatures at the concentrations shown for 90 min, before washing and exposing to CTL clone F8 at a K/T ratio of 5/1 for a 4 h  $^{51}\text{Cr}$ -release assay at 37 °C. Surface expression of D<sup>p</sup> measured with the monoclonal B22.249 after incubation at the different temperatures, but before addition of peptide, was: 9, RMA-S (37 °C); 44, RMA-S (26 °C); 238, RMA (37 °C); 318, RMA (26 °C).

surface expression that is induced by peptide<sup>1</sup>, involves stabilization of empty molecules at the cell surface. Indeed, empty class I molecules can efficiently bind peptide in cell lysates<sup>9,10</sup>. The quantity of peptide taken up by RMA-S through endocytosis is sufficient for stabilization of pulse-labelled H-2 molecules in such lysates<sup>10</sup>. There is therefore no evidence in favour of

retrograde transport of peptide to the ER where it would induce assembly.

Low-temperature induction of heterodimer assembly in the absence of peptide is a novel phenomenon that opens new ways not only to analyse peptide-MHC class I interactions, but also their role in various functions ascribed to MHC class I

FIG. 4 Class I molecules induced at low temperature on RMA-S are not stable at 37 °C but can be stabilized by peptides added exogenously. The influenza peptide NP 365-380, which is a defined epitope presented by D<sup>p</sup> (ref. 29), prevented decay of D<sup>p</sup> induced at 26 °C but not of K<sup>b</sup> (Fig. 4b). The opposite pattern was seen for the peptide NP 345-360, which stabilized K<sup>b</sup> but not D<sup>p</sup> (Fig. 4b). The stabilization lasted for at least 4 h. It was readily observed at a peptide concentration of  $10^{-4}$  M, but was not detectable at concentrations below  $10^{-5}$  M (data not shown). These concentrations of peptide are similar to those required to restore class I cell surface expression in RMA-S at 37 °C (ref. 1). A H-2D<sup>b</sup>-derived sequence (residues 171-182), which competes for H-2D<sup>b</sup>-restricted presentation of peptide<sup>30</sup> could also stabilize D<sup>p</sup> molecules on RMA-S (26 °C) (Fig. 4c). A partial but reproducible stabilization of K<sup>b</sup> was also observed and is consistent with a low affinity of H-2K<sup>b</sup> for the peptide<sup>3</sup>. The decay curves for RMA preincubated at 26 °C and 37 °C converged, suggesting that preincubation at the lower temperature increased the proportion of unstable class I molecules. The data are consistent with the existence of two populations of class I molecules with respect to half-life. One induced at 26 °C on both RMA-S and RMA with a half-life of ~1 h at 37 °C, the other found only on RMA and stable at 37 °C with a half-life > 6 h. a, Decay of H-2D<sup>p</sup> on RMA (left chart) and RMA-S (right chart) preincubated for 24 h at 26 °C (□) or at 37 °C (■), then incubated at 37 °C in the presence of brefeldin A. Cells were stained with monoclonal B22.249 (D<sup>p</sup>, α1) and analysed by FACS (see Fig. 2), at different timepoints after transition to 37 °C. Background staining with



secondary fluorescent antibody alone indicated (---). b, Influenza nucleoprotein-derived peptides prevent decay of induced class I molecules on RMA-S (26 °C). Samples were stained and fixed for FACS analysis (see Fig. 2) after 0, 1, and 4 h at 37 °C, with monoclonals against K<sup>b</sup> (x, 28-13-35) and D<sup>p</sup> (●, 28-14-68). Stabilization generally lasted for at least 4 h. It was readily observed with peptide concentrations of 100 μM or more and was not detected at concentrations below 0.1 μM. c, Peptides derived from a conserved region of the H-2D<sup>b</sup> molecule (residues 171-182) prevent decay of K<sup>b</sup> less efficiently than decay of D<sup>p</sup>. Experimental conditions as in b. METHODS. BFA (2 μl, 5 mg ml<sup>-1</sup> in methanol) was added to a mixture of 0.9 ml of cells in RPMI-1640 and 0.1 ml peptide NP(1934) 365-380 or NP(1934) 345-360, giving a final concentration of 10 μg ml<sup>-1</sup> BFA and 200 μM peptide. In controls, 0.1 ml medium was added without peptide. The mixture of cells, BFA and peptide was then immediately put at 37 °C (5% CO<sub>2</sub>).

molecules. Apart from antigen presentation to class I-restricted CTL<sup>1</sup>, these functions include interactions with other cell surface molecules<sup>11</sup> and target recognition by alloreactive CTL<sup>4,12</sup> and natural killer cells<sup>13,15</sup>.

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## Specific expression of a silk-encoding gene of *Bombyx* in the anterior salivary gland of *Drosophila*

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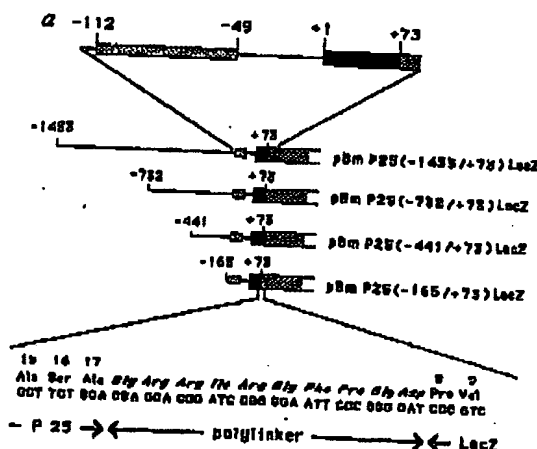
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**SUCCESSFUL** expression of genes transferred into distantly related species in which genetic functions have been maintained through evolution has been reported previously<sup>1-3</sup>. In the case of

the silkworm *Bombyx mori* and the fruitfly *Drosophila melanogaster*, both of which produce chorions (eggshells), *Bombyx* chorion genes are correctly expressed in *Drosophila* despite their estimated 240-Myr phylogenetic divergence<sup>4</sup>. Here we report that, although *Drosophila* does not produce silk, mechanisms regulating transcription have been conserved between the salivary gland of the fruitfly and the silk gland of the silkworm larva.

The P25 gene of *Bombyx mori* is one representative of this set of silk protein genes expressed in the posterior silk gland<sup>4-7</sup>. Different fusion genes were made by joining the *Escherichia coli*  $\beta$ -galactosidase coding sequence to the P25 gene with either 1,453, 732, 441 or 165 base pairs (bp) of P25 5' flanking sequences. In all constructs, the fusion occurred at position +73 of the *Bombyx* gene, 54 bp downstream from the initiation codon (Fig. 1). These sequences were inserted into the vector carnegie 20, which carries the P-element terminal repeats and the gene *rosy* for phenotypic selection of transformants<sup>8</sup>. To account for a possible influence of the *Drosophila rosy* promoter on the

**FIG. 1** P25-lacZ constructs used for *D. melanogaster* transformation experiments and the transformed lines analysed. **a**, Schematic representation of the chimeric genes. P25 genomic fragments with various lengths of 5' upstream sequences (thin line) and the first exon (filled box) were fused in frame with the *E. coli*  $\beta$ -galactosidase coding sequence (hatched box). Numbers represent the distances in nucleotides from the transcription start site. Dotted box, the enhancer element of the P25 gene (J. Dreyer, B. Durand and P.O., unpublished results). The fused genes were inserted into *Drosophila* chromosomes using P-element mediated transformation and *rosy* selection of transformants. **b**, The transformed lines were genetically mapped and made homozygous (H) for the insert, through crosses with balancer stocks. Lethal (L) or sterile (S) insertions were maintained over appropriate balancer chromosomes. All the lines were shown to carry a single copy of the transgene by probing Southern blots with P25 and lacZ DNA. Localization of the insert was determined by *in situ* hybridization to polytene chromosomes with a P25-lacZ probe. Transcriptional orientation of the fused gene was either the same (D) or the opposite (R) to that of the *rosy* gene in the transformation vector injected. **METHODS.** P-element vectors containing P25-lacZ fusion genes were constructed using standard cloning procedures. pBmP25(-1453/+73)lacZ was constructed by sequential insertion in the plasmid pUC18 of the HindIII-PstI (-1453/+73) fragment of the P25 gene and the PstI-PstI 9 kb fragment of the plasmid pMC1671 (ref. 12) containing the lacZ coding region with polylinker sequence. The same procedure was used to construct pBmP25(-732/+73)lacZ, with the HindIII-PstI(-732/+73) fragment. The plasmids containing P25(-441/+73) and P25(-165/+73) sequences were



obtained by partial *Bal*31 digestion of pBmP25(-732/+73)DNA. End points of *Bal*31-digested DNA and the junction regions of P25 to lacZ sequences were ascertained by DNA sequencing. Fragments carrying P25-lacZ constructs were inserted into the *Hpa*I site of the carnegie 20 vector<sup>8</sup>. *Drosophila* recipient strain (ry, sc<sup>sc</sup>) was provided by J. A. Lepoint. Autosomal insertions were made homozygous by crosses with the *CyO*; *TM6*ry<sup>1</sup>/1(2.3)Ap<sup>2</sup> balancer stock. Lines that could not be made homozygous for the transgene were maintained as balanced stocks with *CyO* or *TM6* chromosomes. The X-linked insertion was made homozygous by inbreeding.

TABLE 6. HLA disease associations

Disease	Antigen	Race	Frequency <sup>a</sup>	
			Patients	Controls
Narcolepsy	HLA-DR2	C	1.0	0.22
		O	1.0	0.34
Ankylosing spondylitis	HLA-B27	C	0.89	0.09
		O	0.81	0.01
		N	0.58	0.04
		C	0.47	0.10
Reiter's disease Insulin-dependent diabetes mellitus	HLA-B27	C	0.40	0.21
	HLA-B8	C	0.22	0.14
	HLA-B15	C	0.52	0.22
	HLA-DR3	C	0.74	0.24
	HLA-DR4	C	0.04	0.29
	HLA-DR2	C	0.54	0.27
	HLA-DRB1*0301	C	0.59	0.25
	HLA-DRB1*0401	C	0.85	0.35
	HLA-DQA1*0301	C	0.61	0.23
	HLA-DQB1*0302	C	0.68	0.25
Rheumatoid arthritis	HLA-DR4	C	0.66	0.39
		O	0.44	0.10
		N	0.40	0.32
Hodgkin's disease	HLA-A1	C	0.058	0.013
	HLA-DRB1*1104 <sup>b</sup>	C	0.78	0.28
Hemochromatosis	HLA-A3	C	0.87	0.33
Psoriasis	HLA-Cw6	C	0.79	0.26
Celiac disease	HLA-DR3	C	0.59	0.28
Multiple sclerosis	HLA-DR2	C		

C, Caucasian; O, Oriental; N, Black.

<sup>a</sup>The frequencies given are the total genotypic frequencies of all individuals with at least one copy of the designated allele. Both homozygous and heterozygous individuals are included.

<sup>b</sup>In this case, the frequencies are based on allele frequencies, not genotype frequencies.

Taken from ref. 113.

a more complete retrospective evaluation of the available data suggests that with the exception only of the *H-2K<sup>b</sup>* gene, the spontaneous mutation rate for *H-2* genes was comparable to that for non-*H-2* genes (134). The characterization of these mutant animals, first based on peptide maps and amino acid sequences of the *H-2* proteins (135-138) and later based on the nucleotide sequences of the cloned cDNAs or genes (101,102), provided some of the basic biochemical information on which later studies of structure and function and mechanism of gene evolution were based.

### Expression of MHC Molecules

MHC molecules, synthesized in the ER and destined for cell surface expression, are controlled at many steps before their final disposition as receptors available for interaction with either T cells or NK cells. The MHC-I molecules should be viewed as trimers, consisting of the polymorphic heavy chain, the light chain,  $\beta$ 2-microglobulin, and the assembled self peptide. Since there are numerous steps in the biosynthesis of the MHC-I molecule, regulatory controls can be exerted at almost every step. In addition, reflecting the continuous struggle between the immune system of the vertebrate organism, and rapidly adaptable infectious agents, a number of steps in biosynthesis and expression are inhibited by virus-encoded proteins.

The first level of control of MHC-I expression is genetic; that is, the genes for a particular chain must be present for the trimer to be

expressed. This is of course most relevant for  $\beta$ 2-microglobulin, which is the obligate light chain for the complex. Induced  $\beta$ 2-m-defective animals (*B2m<sup>o</sup>*) (139-141) lack normal levels of MHC-I expression, though for some molecules detectable amounts are present.

The next level of MHC-I expression control is transcriptional, and interferon- $\gamma$  (IFN- $\gamma$ ) regulation is particularly important (142). For the most part, MHC-Ia molecules are ubiquitously expressed, and the basis of the more limited tissue-specific expression of MHC-Ib molecules is only beginning to be explored (143-145). Interest in the regulation of placental HLA-E and HLA-G expression is prompted by a potential role in the mother's tolerance of the fetus.

The rest of the MHC-I biosynthetic pathway is dependent on proper generation of cytosolic peptides by the proteasome and delivery to the ER by TAP, appropriate core glycosylation in the ER, transport through the Golgi, and arrival at the plasma membrane (146). A number of persistent viruses have evolved mechanisms for subverting this pathway of expression. The herpes simplex virus encodes a protein, ICP47, that blocks the activity of the peptide transporter TAP (147-149). Two proteins encoded by the human cytomegalovirus (HCMV), US2 and US11, cause rapid protein degradation of MHC-I molecules, and another HCMV protein, US18, which has sequence similarity to MHC-I molecules, may affect normal MHC-I function by limiting  $\beta$ 2-m availability. The precise mechanism of US18 effects remains controversial. Several viruses, including murine cytomegalovirus (150), adenovirus 2

TABLE 1. Listing of HLA class I alleles

HLA-A		HLA-B		HLA-C		HLA-E		HLA-G	
Serology	Alleles	Serology	Alleles	Serology	Alleles	Serology	Alleles	Serology	Alleles
A1	A*0101,0102	B7	B*0702-0706	Cw1	Cw*0102,0103	—	E*0101-	—	G*01011-
A2	A*0201-0217	B8	B*0801-0803	Cw2	Cw*0201,0202		0104		0104
A3	A*0301,0302	B13	B*1301-1303	Cw3	Cw*0302-0304				
A11	A*1101-1103	B14	B*1401,1402	Cw4	Cw*0401-0403				
A23(9)	A*2301	B18	B*1501-1531	Cw5	Cw*0501				
A24(9)	A*2402-2410	B18	B*1801-1803	Cw6	Cw*0602				
A25(10)	A*2501	B27	B*2701-2710	Cw7	Cw*0701-0705				
A28(10)	A*2801-2808	B35	B*3501-3518	Cw8	Cw*0801-0803				
A29(19)	A*2901,2902	B37	B*3701-3702	—	Cw*12021-1203				
A30(19)	A*3001-3004	B39(18)	B*3801-3802	—	Cw*1301				
A31(18)	A*31012	B39(18)	B*39011-3909	—	Cw*1402,1403				
A32(19)	A*3201	B40	B*40011-4008	—	Cw*1502-1505				
A33(19)	A*3301-3303	B41	B*4101,4102	—	Cw*1601,1602				
A34(10)	A*3401,3402	B42	B*4201,4202	—	Cw*1701,1702				
A36	A*3601	B44(12)	B*4402-4407						
A43	A*4301	B45(12)	B*4501						
A66	A*6601,6602	B46	B*4601						
A68(28)	A*68011-6803	B47	B*4701						
A69(28)	A*6801	B48	B*4801,4802						
A74(19)	A*7401	B49(21)	B*4901						
—	A*6001	B50(21)	B*5001						
		B51(5)	B*5101-5107						
		B52(5)	B*52011,52012						
		B53	B*5301						
		B54(22)	B*5401						
		B55(22)	B*5501-5503						
		B56(22)	B*5601,5602						
		B57(17)	B*5701-5704						
		B58(17)	B*5801,5802						
		B59	B*5901						
		B67	B*67011,67012						
		B73	B*7301						
		B78	B*7801,7802						
		—	B*8101						
		—	B*8201						

This list summarizes the designations of the human MHC-I HLA gene products as they have been known based on serology, and as they have been assigned by nucleotide (and thus inferred amino acid) sequences. This table is taken from that of McCluskey (262). Frequently updated listings of HLA alleles as well as alignments of their sequences can be found at: <http://www.icsnet.uk/arp/tia>. Current serologic designations are given in the "serology" column, with older corresponding numbers listed in parentheses. It is apparent that some of the most recently identified alleles (in particular, those of HLA-E and HLA-G) have not been identified serologically.

chain. The heterodimers usually consist of the assembled products of the linked genes encoding the two chains. In the mouse, the products of the *IAs* (also known as *IA $\alpha$* ) and *IAb* (or *IA $\beta$* ) genes assemble to form the IA heterodimer, and similarly, the products of the *IE $\alpha$*  (*IE $\alpha$* ) and *IE $\beta$*  (*IE $\beta$* ) genes assemble to form IE. IA and IE are often referred to as *iso*types. The allelic forms are usually referred to as *IA<sup>b</sup>*, *IA<sup>a</sup>*, or *IA<sup>k</sup>*. Under some circumstances, mixed heterodimers, which can be of immunologic importance, are observed (23-27). Thus, if one is referring to a mixed heterodimer consisting of the  $\alpha$  chain of *IE<sup>a</sup>* and the  $\beta$  chain of *IA<sup>b</sup>*, one must use the more precise but cumbersome description *IA $\beta$ <sup>a</sup>IE $\alpha$ <sup>b</sup>* (*IA $\beta$ <sup>a</sup>IE $\alpha$ <sup>b</sup>*). In the human, particularly in referring to MHC-II molecules, the distinctions between molecules identified by antibodies and those identified by DNA sequence typing must be made (see Tables 1 and 2). The bulk of the serologically defined differences rest with the  $\beta$  chain.

### The Function of MHC Molecules

The major function of the molecules encoded by the *Mhc* is to facilitate the display of unique molecular fragments on the surface of cells in an arrangement that permits their recognition by immune effectors such as T-lymphocytes. The MHC-I or MHC-II molecules are those cell surface glycoproteins that actually perform the binding and recognition steps, while other genes that map to the *Mhc-I* or *Mhc-II* regions may contribute to antigen-processing and -presentation functions in other distinct ways. The MHC molecule accomplishes its major role in immune recognition by satisfying two distinct molecular functions: the binding of peptides (or in some cases nonpeptidic molecules) and the interaction with T cells, usually via the  $\alpha\beta$  T-cell receptor (TCR). The binding of peptides by an MHC-I or MHC-II molecule is the selective event that permits the cell expressing the MHC molecule (the antigen-

TABLE 2. Listing of HLA class II alleles

HLA-DR		HLA-DQ		HLA-DP	
Serology	Alleles	Serology	Alleles	Serology	Alleles
<b><math>\alpha</math>-Chain</b>					
	DRA*0101-0102		DQA1*0101-0105		DPA1*0103-0104
			DQA1*0201		DPA1*0201-0202
			DQA1*0301-0303		DPA1*0301
			DQA1*0401		DPA1*0401
			DQA1*0501-0503		
			DQA1*0601		
<b><math>\beta</math>-Chain</b>					
DR1	DRB1*0101-0104	DQ5(1)	DQB1*0501-0504	DPw1	DPB1*0101
DR15(2)	DRB1*1501-1505	DQ6(1)	DQB1*0601-0611	DPw2	DPB1*0202-0202
DR16(2)	DRB1*1601-06	DQ2	DQB1*0201-0203	DPw3	DPB1*0301
DR3	DRB1*0301-0308	DQ3	DQB1*0301-0306	DPw4	DPB1*0401-0402
DR4	DRB1*0401-0423	DQ4	DQB1*0401-0402	DPw5	DPB1*0501
DR11(5)	DRB1*1101-1127			DPw6	DPB1*0801
DR12(5)	DRB1*1201-1204			—	DPB1*0801-4101
DR13(6)	DRB1*1301-1312			—	DPB1*4401-6501
DR14(6)	DRB1*1401-1425				
DR7	DRB1*0701				
DR8	DRB1*0801-0813				
DR9	DRB1*0901				
DR10	DRB1*1001				
	DRB3*0101				
DR52	DRB3*0201-0205				
	DRB3*0301				
DR53	DRB4*0101-0103				
	DRB4*0101-02N				
DR51	DRB5*0101-0105				
	DRB5*0201-0203				

Lists of HLA alleles and regular updates and aligned sequences can be assessed from the TAL Homepage at: <http://www.icsnet.uk/axp/tla>

As illustrated by this table, the serologic assignments of HLA class II molecules do not always correlate with the DNA nomenclature. Serologic assignment of HLA-DR molecules is largely determined by the DRB1 gene product, while assignment of DQ molecules reflects serologic contributions from both DQA1 and DQB1 gene products. As new alleles of DR and DQ have been identified, the assignments have been "split." Thus, DR15 and DR16 are splits of DR2, DR11, and DR12 are splits of DR6, and so forth. The "w" designations (for HLA-C and HLA-DP) are "workshop" assignments because the serologic assignments have been imprecise.

presenting cell, APC) to sample either its own proteins (in the case of MHC-I) or the proteins ingested from the immediate extracellular environment (in the case of MHC-II). In particular, cell surface MHC class I glycoproteins gather from the cell's biosynthetic pathway fragments of proteins derived from infecting viruses, intracellular parasites, or self molecules, either normally expressed or dysregulated by tumorigenesis, and then display these molecular fragments at the cell surface (7,9,28). Here the cell-bound MHC-I-peptide complex exposed on the APC is displayed to T cells. The second characteristic of the MHC-I molecule, the ability to interact with TCR, then allows the APC bearing a particular MHC-peptide complex to engage an appropriate  $\alpha\beta$  TCR as the first step in the activation of a cellular program that might lead to cytotoxicity of the APC as a target and/or to the secretion of lymphokines by the T cell. The interaction with the TCR is dependent on both the peptide and the MHC molecule. As a rule, a specific TCR will not bind the MHC molecule alone or when complexed with an unrelated peptide. Some would argue that the major evolutionary basis for the development of such a system is to discriminate those cells of the host that are infected by viruses or other obligate intracellular parasites (28). Thus, a system that originally evolved for identifying cells afflicted by viruses or other intracellular parasites might then

also function to identify antigens specific to tumor cells (29). For MHC-I restricted antigens, the usual rule is that the peptides are generated in the same cell that synthesizes the MHC-I molecule. Generally speaking, these peptides derive from proteins found in the cytosol that are then degraded by the multiproteolytic proteasome complex into peptides, and the resulting peptides, transported from the cytosol to the endoplasmic reticulum with the aid of the intrinsic membrane transporter, the transporter associated with antigen processing (TAP), are then cooperatively folded into the newly synthesized MHC-I molecule (30).

Exploiting similar molecular mechanisms, MHC class II molecules bind peptides derived from the degradation of proteins ingested by MHC-II-expressing APC, and display them at the cell surface for recognition by specific T-lymphocytes. The MHC-II antigen presentation pathway is based on the initial assembly of the MHC-II  $\alpha\beta$  heterodimer with a dual function molecule, the invariant chain (Ii) that serves as a chaperone to direct the  $\alpha\beta$  heterodimer to an endosomal, acidic protein-processing location where it encounters antigenic peptides, which also serves to protect the antigen-binding site of the MHC-II molecule so that it preferentially will be loaded with antigenic peptides in this endosomal-lysosomal location (31,32). The loading of the MHC-II mole

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TABLE 3. Commonly used mouse strains: H-2 haplotypes<sup>a</sup>

Strain	Haplotype	H-2 complex							
		K	Ab	Aa	Ed	Ea	D	Qa1	Tla
Common strains									
129/J	bc	b	b	b	b	—	b	b	f
AKR/J	k	k	k	k	k	k	k	b	b
A.SW/Sn	s	s	s	s	s	—	s	b	b
BALB/cJ	d	d	d	d	d	d	d	b	c
C3H/HeJ	k	k	k	k	k	k	k	b	b
CBA/J	k	k	k	k	k	k	k	b	b
C57BL/6	b	b	b	b	b	—	b	b	b
C57BL/10	b	b	b	b	b	—	b	b	b
C57BR	k2	k	k	k	k	k	k	s	a
DBA/2J	d	d	d	d	d	d	d	b	c
NZB/BINJ	d2	d	d	d	d	d	d	s	a
P/J	p	p	p	p	p	p	p	s	a
RHIS/J	r	r	r	r	r	r	r	c(r)	b
SJL	s2	s	s	s	s	—	s	a	a
Congenic strains									
B10.BR	k2	k	k	k	k	k	k	s	a
B10.D2	d	d	d	d	d	d	d	b	c
B10.S	s	s	s	s	s	—	s	b	b
BALB.B	b	b	b	b	b	—	b	b	b
BALB.K	k	k	k	k	k	k	k	b	b
C3H.SW	b	b	b	b	b	—	b		
Recombinant strains									
A	a	k	k	k	k	k	d		
ATL	t1	s	k	k	k	k	d		
B10.A	a	k	k	k	k	k	d		
B10.A(1R)	h1	k	k	k	k	k	b		
B10.A(2R)	h2	k	k	k	k	k	b		
B10.A(3R)	i3	b	b	b	b/k	k	d		
B10.A(4R)	h4	k	k	k	k/b	—	b		
B10.A(5R)	i5	b	b	b	b/k	k	d		
B10.T(6R)	y2	q	q	q	q	—	d		
B10.S(7R)	i2	s	s	s	s	—	d		
B10.B(8R)	as1	k	k	k	k/s	—	s		
B10.B(9R)	i4	s	s	s	s/k	k	d		
B10.HTT	i3	s	s	s	sd/k	k	d		

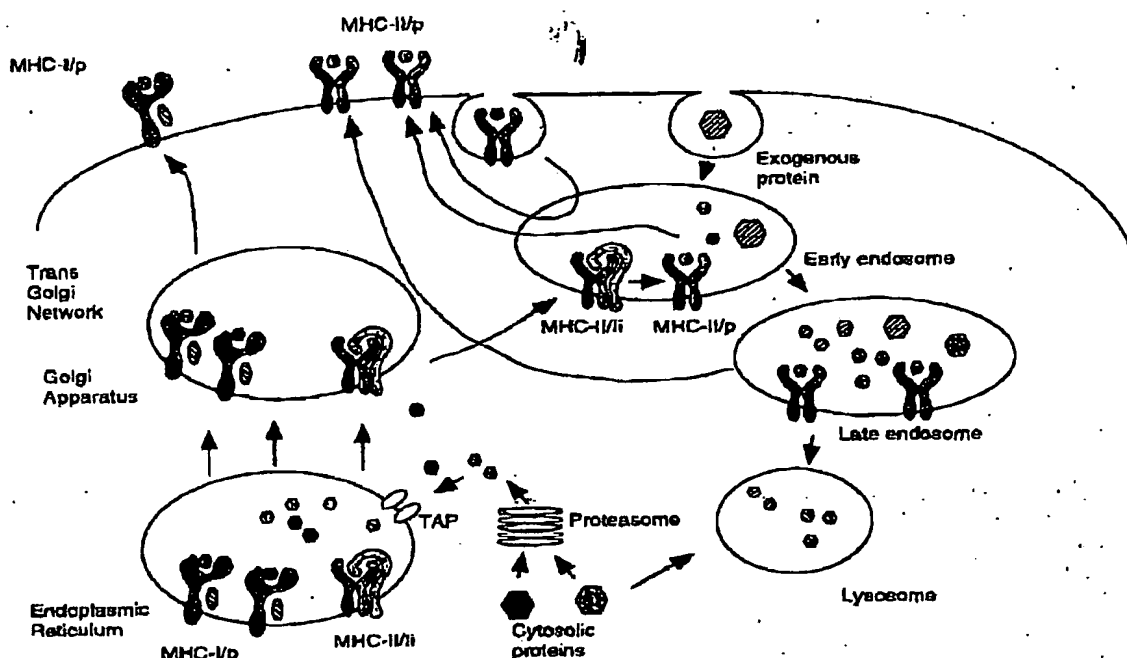
<sup>a</sup>A dash indicates that the gene at that locus is not expressed normally, though the precise mechanism in different strains may differ (270). Several designations, such as bc, a2, and k2, follow the suggestion of Lindahl (18) to clarify differences in the genotype in some of the distal MHC loci.

cule with antigenic peptide, a process dependent on the release of the li-derived CLIP peptide, in part dependent on the MHC-II-like molecule, HLA-DM in the human (33,34), then leads to the cell surface expression of MHC-II peptide complexes. The MHC-II-recognizing T cells then secrete lymphokines and may also be induced to proliferate. Although these cell surface MHC molecules have been named for their strong effects in tumor and tissue transplantation across genetic barriers, their molecular and cellular function is more general, and it is perhaps better to think of MHC-I or MHC-II as the names of the peptide receptor on the APC.

MHC-I and MHC-II molecules, because of differences in their protein structure and the resulting differences in the cellular compartments that they traverse from their biosynthesis to their maturation, have strong preferences for the origin of the proteins that they sample for antigen presentation (35,36). The MHC-I antigen presentation pathway is most easily thought of as an inside-out pathway by which protein fragments of molecules synthesized by

the cell are delivered to and bound by the MHC-I molecule during its biosynthesis. In contrast, the MHC-II antigen presentation pathway is best more clearly visualized as an outside-in one in which ingested proteins are degraded by enzymes in the endosomal-lysosomal system and are delivered to the MHC-II molecules in that degradative compartment (37). These processes are schematically illustrated in Fig. 1 and are described in more detail elsewhere in this volume. The biochemical steps involved in the production of antigen fragments from large molecules are collectively known as *antigen processing*, while those that concern the binding of antigen fragments by MHC molecules and their display at the cell surface are known as *antigen presentation*. In general, the antigen-processing and -presentation pathways have been described experimentally for peptide antigenic fragments derived from proteins, and processing is a series of events focused on identification of the dys-regulated or foreign protein and its proteolytic degradation into short peptides. Presentation consists of the binding of the peptide

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**FIG. 1.** Antigen processing and presentation. The major pathways of processing and presentation are shown. Cytosolic proteins (*shaded hexagons*) are degraded in proteasomes to peptide fragments that are then transported into the endoplasmic reticulum by TAP, where they assemble with MHC-I/β2-m complexes. From there they pass through the Golgi and trans Golgi network to the cell surface. Exogenous proteins (*striped hexagons*) enter the endosomal pathway, and in early or late endosomes, or lysosomes, they are progressively degraded to peptides. In early and late endosomes, MHC-II-invariant chain complexes (that have been trafficked there from the endoplasmic reticulum) are converted to MHC-II/CLIP complexes that are loaded with peptides with the catalytic aid of HLA-DM. These MHC-II-peptide complexes then go to the plasma membrane. (Adapted from refs. 37 and 148.)

fragment by the MHC-I or MHC-II molecule and the subsequent movement to the cell surface for display to the extracellular environment.

In addition to showing preference to distinct pathways of antigen presentation, the MHC-I and MHC-II molecules also show preferential restriction to T cells of the CD8- or CD4-bearing subsets. This is related to the observation that CD8 binds to the nonpolymorphic α3 domain of MHC-I molecules (38–41), while CD4 interacts with membrane proximal domains of MHC-II (42–44). The CD8 and CD4 molecules serve as coreceptors on the surface of the T-lymphocyte, providing both adhesion (avidity increase) and specific activating signals that modulate the avidity of the T cell in a time-dependent manner (45). Table 4 summarizes the similarities and differences between MHC-I and MHC-II molecules.

As high-resolution maps of the *Mhc* are developed, it has become clear that a number of molecules with function related to antigen presentation, but not necessarily the antigen-presenting molecules themselves, also map to the *Mhc*. These include the H-2M and HLA-DM molecules of the mouse and human, which are MHC-II-like in amino acid sequence, but which appear to play a catalytic role in augmenting the binding of peptides to MHC-II molecules in the acidic endosomal or lysosomal compartment of

the APC (46). The *TAP1* and *TAP2* genes encode molecules that are related to those of the ATP-binding cassette transporter family (47), which are important in the delivery of peptides generated in the cytosol to the nascent MHC-I molecule as it assembles with its light chain β2-m.

An additional function of MHC molecules that is not directly related to T-cell recognition has been recognized in recent years. This is to serve as elements for signal transduction to natural killer (NK) cells. NK cells are non-T lymphoid cells that are capable of lysing some tumor cells' targets and some cells infected with intracellular pathogens through a process known as natural killing. The expression by the target cell of MHC class I molecules can, in certain cases, protect the target from killing by the NK effector, and target cells defective in the expression of normal MHC class I cells are susceptible to such NK-cell lysis (11,48). In some other cases, receptors on NK cells that interact with MHC-I are activated by the interaction. Studies have revealed several families of activating and inhibitory receptors in the human, rat, and mouse. These NK receptors, in addition to falling into roughly two classes of inhibitory receptors or activating receptors, also, by their structure, can be classed as either those that belong to the immunoglobulin supergene family or those that belong to the C-type lectin family (49).

TABLE 4. Comparison of MHC-I and MHC-II molecules

	MHC-Ia and Ib	MHC-II
Genetics	Multiple heavy-chain loci, most linked to the MHC Light-chain, $\beta$ 2-m is genetically unlinked.	Several heavy- and light-chain loci, $\alpha$ - and $\beta$ -chain genes linked to each other
Tissue-specific expression	MHC-Ia, ubiquitous	MHC-II on B cells, macrophages, dendritic cells, Langerhans cells (in the mouse); in human, also found on T cells and many activated cell types
Molecular structure	Heavy-chain-light-chain form heterodimer. Obligate cell-surface molecule. Heavy chain has three extracellular domains, $\alpha$ 1, $\alpha$ 2, and $\alpha$ 3. $\alpha$ 1/ $\alpha$ 2 form peptide binding site; $\alpha$ 3 and $\beta$ 2-m are Ig-like. Only heavy chain is membrane-bound, $\beta$ 2-m is noncovalently assembled.	$\alpha$ and $\beta$ chains form heterodimer of four domains; $\alpha$ 1/ $\beta$ 1 form peptide binding site; $\alpha$ 2 and $\beta$ 2 are Ig-like. Both chains are membrane-bound. Association of nascent MHC-II with invariant chain.
Site of peptide acquisition	1. In endoplasmic reticulum during biosynthesis 2. At cell surface when exposed to exogenous peptides	In endosome or lysosome where degraded products of ingested proteins are encountered; binding of peptides mediated by H-2M (in mouse) or HLA-DM (human).
Nature of peptides bound	MHC-Ia preferences for 8 to 10mers; though longer peptides can be bound. "motif" residues for particular MHC-I molecules CD1 capable of binding lipid antigens	Longer peptides are acceptable.
T-cell recognition	Primarily CD8 <sup>+</sup>	Primarily CD4 <sup>+</sup>
Associate molecules	$\beta$ 2-microglobulin TAP Tapasin Calnexin	li-invariant chain H-2M, (HLA-DM)
Alternate functions	Interaction with NK receptors As nFcr, binding Fc	Interactions with NK receptors

## THE MAJOR HISTOCOMPATIBILITY COMPLEX

### Mhc Genetic Maps

The MHC is an extended region of the genome that spans some 4 million basepairs (bp) on the short arm of human chromosome 6 between 6p21.31 and 6p21.32. In the mouse, the MHC occupies a central region of about 2 cM of chromosome 17 that extends from 18.0 to 20.0 cM. Although this region has not yet been contiguously physically mapped in the mouse, it is likely that it will extend for about the same distance (19). Genome mapping and sequencing have identified numerous genes in this region, many of which have functions critically related to those of the MHC class I or class II proteins, others of which have immunologically relevant functions as well. However, a large number of genes that map to this region also seem to have very little in common with immunologic function. Figure 2 shows schematically a map of some of the major genes of the human, mouse, and rat *Mhc*, which includes those that encode MHC-I and MHC-II proteins. These comparative maps are not drawn to scale and do not show every gene identified in the region. Three markers, *Kc3*, *Bal1*, and *Mog* serve to define the gross colinearity of the MHC of the three species (50), though there are clearly major differences between strains and individuals within a species and between the species as well. The mapping information now available for the human is more extensive than that available for the mouse, and the rat lags behind the other species. A database of the human *Mhc* is available via the World Wide Web (<http://www.hgmp.mrc.ac.uk>) (51). A map showing the homology of mouse chromosome 17 to the human can be found at (<http://www3.ncbi.nlm.nih.gov/Homology/mouse17.html>), and a YAC map of the human *Mhc*-

I region is at (<http://chimera.biotech.washington.edu/UWGC/projects/his-1/HLAysa.htm>). One tabulation counts 212 genes in 491 loci that have been identified (51). With the continuing progress of the human genome project, we can expect that the full sequence of the 4,000 kbp of the human *Mhc* will be completed and the precise identification of open reading frames there will be made. Because of its importance as a model system, the linear sequence of the *Mhc* of the mouse should also be completed.

The human MHC map reveals clusters of genes grouped roughly into an *Mhc* class II region covering about 1,000 kb, an *Mhc* class III region, and an *Mhc* class I region (see Fig. 2). *HLA-DP* genes (*DPA* encoding the  $\alpha$  chain, and *DPB* encoding the  $\beta$  chain) are proximal to the centromere on the short arm of the chromosome and are linked to the genes encoding the related HLA-DM molecule (*DMA* and *DMA*). Between these and the *DQ* genes lie *LMP* genes (for low-molecular-weight proteins (52–55)) and *TAP* (56–60) (for transporter associated with antigen-processing) genes. *LMP* and *TAP* genes encode molecules that are involved in peptide generation in the cytosol and peptide transport across the endoplasmic reticulum (ER) membrane, respectively. The *TAP* genes encode a two-chain intrinsic membrane protein that resides in the ER of all cells, and functions as an ATP-dependent transporter that pumps peptides generated in the cytosol into the lumen of the ER (47). The current view of the LMPs is that they are subunits of the multicatalytic proteolytic proteasome complex that regulate the specificity of cleavage of proteins, and thus modulate the repertoire of peptides available for MHC-I restricted antigen presentation (61, 62). An elegant description of the selective transport of cytoplasmically generated peptides by different TAP proteins in the rat demonstrates that the spectrum of MHC-peptide complexes

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